Determination of Carbetamide Residues and Its Aniline Metabolite

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Carbetamide [D(−) - phenylcarbamoyloxy - 2 - (*N*-ethylpropionamide)] is a selective herbicide which controls weeds in alfalfa and cereals by preemergence and/or postemergence applications. The parent compound and its aniline metabolite were separated by liquid–liquid partition of the crop extract. After acid hydrolysis the carbetamide afforded quantitative yields of ethylamine and aniline which were converted to the corresponding amides with 4-bromobenzoyl chloride. The amides were then separated by column chromatography on Florisil. The limits of detection of these derivatives by electron-capture gas chromatography were generally less than 0.05 ppm and in some cases less than 0.02 ppm.

Carbetamide [D(−) - phenylcarbamoyloxy - 2 - (*N* - ethylpropionamide)] is an experimental selective herbicide produced by Rhone-Poulenc, France. Since the compound is being considered for the control of certain weeds in cereals, wheat, barley, forages, and alfalfa by preemergence and/or postemergence applications, a method was needed to determine the residues of this material. In studying its metabolism, Desmoras *et al.* (1967) found that aniline was present in plants in small quantities soon after treatment with the parent compound, but was rapidly eliminated. The degradation of carbamates in soil and crops has been extensively studied (Kaufman, 1967, for leading references) and aniline derivatives have been reported as metabolites of phenyl carbamates. Tilden and Van Middlelem (1970) succeeded in determining carbaryl as the amide derivative with electron-capture gas chromatography. The object of the present investigation was to develop a method that would be specific for carbetamide and at the same time detect the aniline metabolite.

EXPERIMENTAL

Reagents and Solvents. The acetone, chloroform, methylene chloride, benzene, and ethyl acetate were all nanograde quality and obtained from the Mallinckrodt Chemical Works, Saint Louis, Mo. The ethylamine hydrochloride was "high purity," the aniline analytical grade reagent, and both were obtained from the J. T. Baker Chemical Co., Phillipsburg, N.J. The 4-bromobenzoyl chloride was obtained from Eastman Organic Chemicals, Rochester, N.Y., and the "Hyflo" Super-Cel was obtained from Johns-Manville, Manville, N. J. The Florisil PR grade was purchased from the Floridin Company, Berkeley Springs, W. Va.

Sample Preparation. "P" grams of finely chopped, frozen crop, P/2 g of anhydrous sodium sulfate, and then *ca.* 50 g of Dry Ice were ground in a Waring blender to afford a powdery mixture. The Na_2SO_4 was not required if the sample was fairly dry. One hundred to 150 ml of acetone was added and the mixture well blended at high speed for 1–2 min. P may be equal to 25 g of dry straw, 50 g of alfalfa, pea pods, and vines, or 100 g of wheat or barley grain. The homogenates were filtered under vacuum through a Buchner funnel containing a deionized water-moistened Whatman No. 1 filter paper on which was a pad of glass wool and a layer of "Hyflo" Super-Cel. After washing the filter cake with about 150 ml of acetone and sucking it dry, the cake was reblended with 100 ml of acetone for 1–2 min and then filtered. The blender was washed with 100 ml of acetone and the washings and extracts were combined.

Separation of Free Aniline from Carbetamide. The extract was transferred to a 1-l. separatory funnel, and 250 ml of methylene chloride and 70 ml of a 20% solution of sodium chloride in 1 *N* HCl were added. The mixture was well shaken and the layers were permitted to separate. This separation proceeded slowly. The aqueous phase was separated and the organic phase extracted with 40 ml of the acidic sodium chloride solution. The aqueous phases were combined, transferred to a 500-ml separatory funnel, and washed with 50 ml of methylene chloride. The separated aqueous layer was put aside. The methylene chloride extracts were combined, filtered, and then evaporated under vacuum in a rotary evaporator, with a bath temperature below 40°C. The residue, which may contain 5–10 ml of water, was quantitatively transferred to a 250-ml separatory funnel. The evaporation flask was washed with 40 ml of 1 *N* HCl and then 50 ml of methylene chloride, and the combined washings were slowly poured into a separatory funnel. The mixture was shaken gently (to avoid emulsion formation), the phases were allowed to separate, and the organic layer was filtered through a Whatman No. 1 filter paper containing several grams of anhydrous sodium sulfate. The aqueous layer was extracted three more times with methylene chloride. The combined filtered organic phases were evaporated under vacuum in the rotary evaporator, with a water bath temperature below 40°C (Extract A). The aqueous acidic extracts were combined with the previously separated aqueous layer (Extract B).

Hydrolysis and Digestion of Carbetamide. To the organic extract (Extract A) was added 5 ml of 8 *N* H_2SO_4 and the mixture heated under reflux for 2 hr at about 120°C. After cooling to room temperature the condenser was flushed with 30 ml of water into the main fraction. Twenty-five milliliters of 10 *N* NaOH was added to the hydrolysate to afford a pH >10. Two drops of silicone antifoam were added and the mixture was distilled until 30 to 40 ml of distillate were collected. The distillates containing aniline and ethylamine were acidified to pH 4–5 with 5 ml of 0.1 *N* HCl and then transferred to a 250-ml separatory funnel. The acidified aqueous phase was extracted twice with 25-ml portions of $HCCl_3$ and then the organic layer discarded.

Derivatization of Ethylamine and Aniline. To the acidified hydrolyzed aqueous extract was added 25 ml of benzene followed by 10 ml of a 0.1% solution of 4-bromobenzoyl chloride in benzene. Cautiously, 5 ml of 5 *N* NaOH was introduced by pipette into the aqueous layer to afford a pH of at least 10. The flask was quickly stoppered and the mixture stirred magnetically for 15–20 min at room temperature. The two phases were separated in a separatory funnel and the

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benzene layer was filtered through anhydrous sodium sulfate. The aqueous phase was extracted three times with 50-ml portions of benzene and the combined organic fractions were then evaporated under vacuum on the rotary evaporator with a water bath temperature under 40°C.

Distillation and Derivatization of Free Aniline. The combined aqueous acidic extracts (Extract B) were made basic to pH >10 with 30 ml of 10 *N* NaOH and immediately distilled until 50–60 ml was collected as described above. The distillate was then acidified, extracted, and derivatized as described above.

Florisil Chromatography of the Amide Derivatives. A glass column 2.5 × 50-cm containing a coarse porosity fritted disc and fitted with a Teflon stopcock was packed with a small wool plug. The column was filled with 100 ml of benzene, 25 g of Florisil added, and then mild suction applied. The packed column was washed with about 100 ml of benzene and then a layer of anhydrous Na_2SO_4 followed by a glass wool plug was placed on top of the absorbent. The amide extracts were quantitatively transferred to the column with 5 to 10 ml of benzene. The aniline amide was eluted with 250 ml of 2% ethyl acetate in benzene. The receiver flask was changed and the ethylamine amide was eluted with 250 ml of 10% ethyl acetate in benzene. The rate of elution was maintained at about 60 drops per min. The eluates were concentrated under reduced pressure to afford a volume which would permit a suitable residue concentration for the ensuing glc determination.

Gas Chromatography. A Micro-Tek Model MT 220 (Tracor Analytical Instruments, Austin, Texas) gas chromtograph equipped with a ^{63}Ni electron-capture detector was used for the determination of the derivatized residues. This instrument was fitted with a 4-ft × 6-mm o.d. glass column packed with 5% OV-210 silicone fluid on 80/100 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) The following operating parameters were generally used: 5% methane in argon at 110 ml/min at 40 psi; column/oven temperature 220°C; injection port 250°C; detector temperature 280°C; recorder chart speed 0.25-in. per min. Under these conditions the retention times of 4-bromo-*N*-ethylbenzamide and 4-bromo-*N*-phenylbenzamide were 1.5 and 6 min, respectively. The concentrations of the solutions were adjusted so that when a 1-μl sample was injected, it corresponded to concentrations located within the linear range of the standard curves. The height (H) in mm of each sample peak was measured and compared with that (h) of a 1-μl injection corresponding to C nanograms per microliter of the reference standards. The unknown quantity (X) of sample injected in nanogram per microliters equivalent to micrograms per milliliter (μg/ml) was $X = \mu g/ml = HXC/h$. Thus, ppm = (μg/ml) × sample dilution in ml/sample weight in grams, where X = unknown concentration of sample in μg/ml, C = known concentration of reference standard in μg/ml, H = peak height of sample in mm, and h = peak height of reference standard in mm corresponding to concentration C.

Preparation of Standard Solutions. One-tenth gram of carbetamide (min. 99.0% pure) and aniline were dissolved in 1 l. of acetone and 1 l. of 0.1 *N* HCl, respectively. Since hydrolyzed carbetamide yields 19% ethylamine (w/w), a 19% aqueous solution of ethylamine was also prepared. The carbetamide solution was subjected to hydrolysis followed by steam distillation, derivatization of the resultant ethylamine, and aniline and then Florisil chromatography. The aniline derivative (first fraction from the column) was diluted to afford concentrations of 0.1, 0.2, 0.5, 0.75, and 1.0 μg per ml equivalents of carbetamide. The ethylamine derivative (second fraction from the Florisil column) was diluted to afford 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 μg per ml equivalents of carbetamide. The aniline stock solution was subjected to steam distillation and derivatization as described above. After Florisil chromatography the aniline derivative was diluted to afford 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 μg per ml equivalents of aniline. The quantitative conversion of carbetamide to ethylamine and aniline was confirmed by comparison of the peak height of the liberated ethylamine with that of the ethylamine standard.

SUMMARY AND DISCUSSION

The parent compound and the free aniline were separated by liquid–liquid partition between methylene chloride and an acidic aqueous phase. The solvent phase containing the carbetamide was evaporated and the residue submitted to acid hydrolysis and digestion with 8 *N* H_2SO_4. This hydrolysis afforded quantitative yields of ethylamine after refluxing the mixture for 2 hr. Aniline formation was quantitative after only 30 min. The acidic hydrolysate was made basic and steam distilled. The condensed distillates containing ethylamine and aniline were treated with base and converted to the amide derivatives by coupling with 4-bromobenzoyl chloride and then separated on the Florisil column. The free aniline derivative was obtained by treating the distillate containing aniline with base, followed by treatment with 4-bromobenzoyl chloride. During the hydrolysis of the carbetamide, the acid oxidizes the plant extractives, converting them to water-soluble products. Tilden and Van Middlelem reported that additional clean-up takes place during the ensuing steam distillation and later during the coupling reaction and ensuing liquid–liquid partition. Final purification was achieved by Florisil column chromatography (Figure 1). Recoveries of carbetamide and its metabolite from spiked crops at the extraction step varied from 78.0–98% calculated from the ethylamine derivative and from 78.0–91.0% calculated from the aniline derivative as shown in Table I. Typical chromatograms obtained are shown in Figure 2.

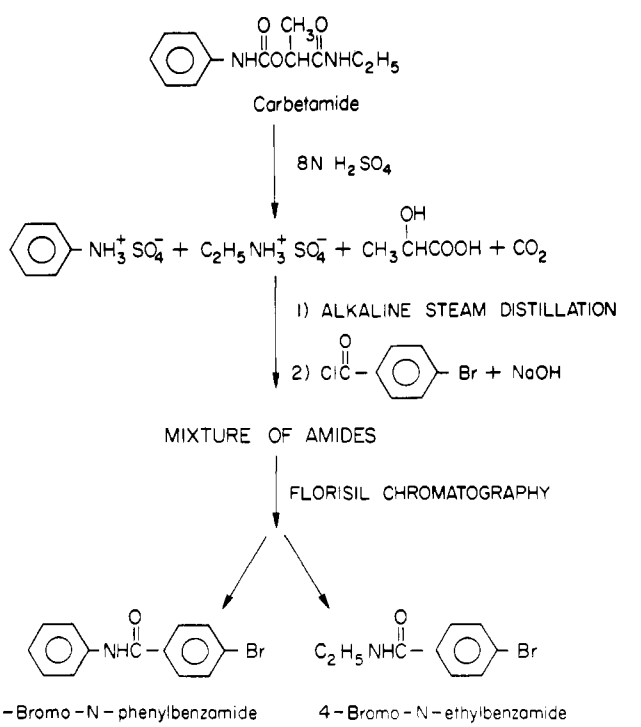

Figure 1. Separation and derivatization of carbetamide hydrolysate

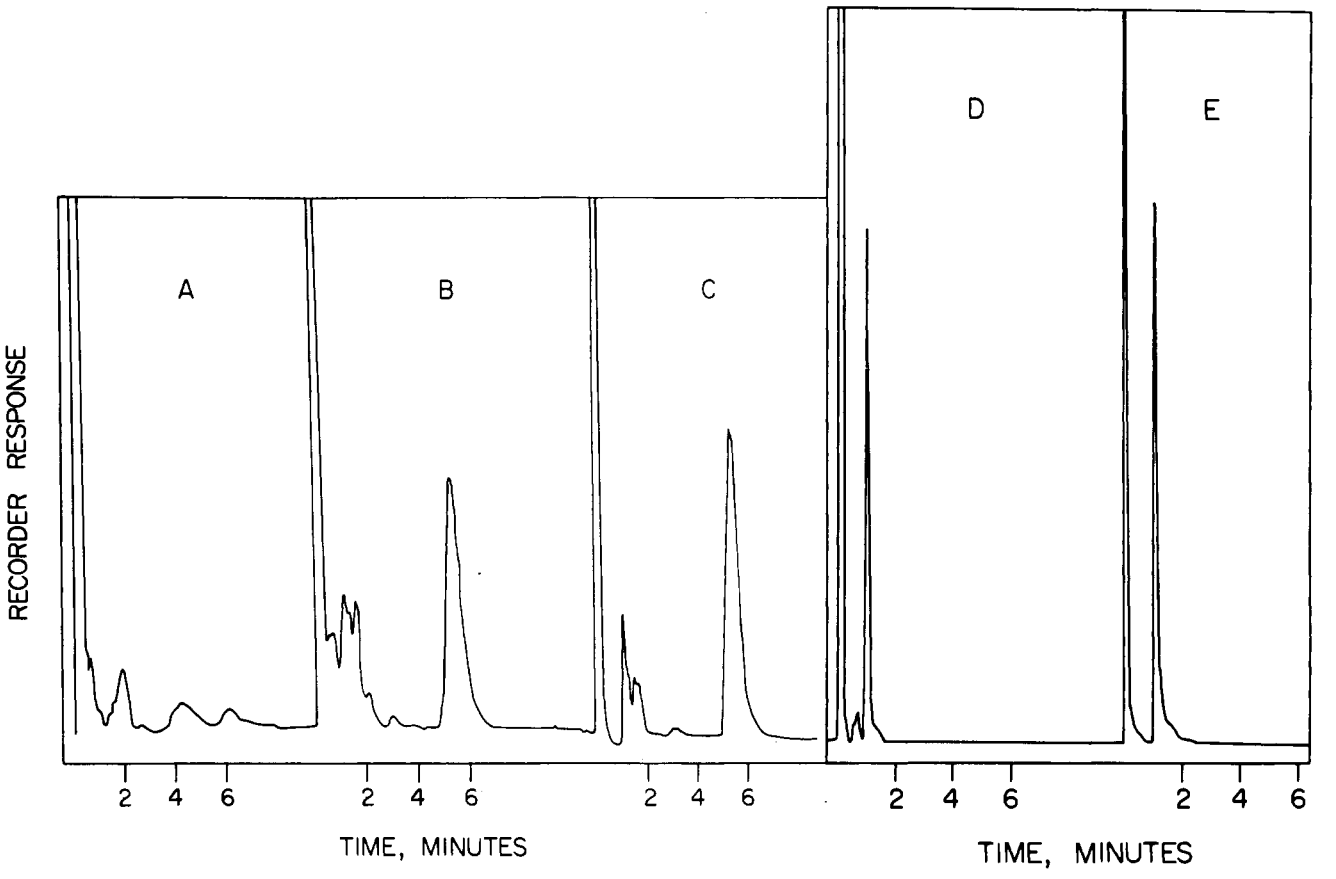

Figure 2. Typical carbetamide recoveries from spiked alfalfa. A. Glc of untreated alfalfa. B. Alfalfa containing 0.2 ppm of carbetamide. Peak at 6 min corresponds to 0.415 μg/ml of carbetamide equivalent. Recovery 83%. C. 4-Bromo-*N*-phenylbenzamide standard, equivalent to 0.5 μg/ml of carbetamide. D. Alfalfa containing 0.2 ppm of carbetamide. Peak at 1.5 min corresponds to 0.184 μg/ml of carbetamide equivalent. Recovery 92%. E. 4-Bromo-*N*-ethylbenzamide standard, equivalent to 0.2 μg/ml of carbetamide

Table I. Recoveries of Carbetamide and Aniline Added to Untreated Alfalfa, Wheat, Barley, and Pea Vines

Crop and sample no.	Added, ppm	Carbetamide equivalent % recovery calculated from: Ethylamine derivative	Aniline derivative	Aniline equivalent added, ppm	Recovery %
Alfalfa #917	0.20	94.0	84.0		
Alfalfa #917	0.50	85.0	87.0	0.50	80.0
Pea vines #1016	1.00	98.0	81.0		
Wheat grain #1014	0.10	97.0			
Wheat grain #1014	0.10	97.0		0.10	86.0
Wheat grain #1014	0.40		91.0	0.25	82.0
Wheat grain #1014	0.50	82.0	82.0		
Wheat straw #1014	0.40	85.0	85.0	0.40	85.0
Barley straw #1012	1.00	78.0	78.0		
Averages		89.5	84.0		83.3

Table II. Theoretical Detectable Levels of Crops Spiked with Carbetamide and Aniline

Sample dilutions, g/ml	Carbetamide, equivalents, ppm: Aniline derivative	Ethylamine derivative	Aniline equivalents, ppm aniline derivative
25/10	0.04	0.020	0.020
50/25	0.05	0.025	0.025
100/50	0.05	0.025	0.025

In practice, the sensitivity of the method was limited by the findings in control samples of impurity peaks emerging at or near the retention times of interest. However, the method described had sufficient clean-up potential to maintain the apparent calculated findings in the controls to a theoretical minimum (<0.05 ppm to <0.02 ppm).

The minimum detectable values given in Table II were obtained by diluting 25, 50, or 100 g of crop substrate with solvent to afford total volumes of 10, 25, or 50 ml and were calculated from the aniline or ethylamine derivatives minimum detectable peak heights. The minimum detector responses of the aniline and ethylamine derivatives were, respectively, 0.1 and 0.05 μg/ml carbetamide equivalents. The linearity range was at least ten times greater than the working concentrations.

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